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(54) Title: COMPOSITIONS AND METHODS FOR TISSUE REPAIR

(57) Abstract

Compositions comprising at least one structural biomaterial, e.g., polypropylene mesh, integrated with at least one biodegradable matrix, e.g., collagen glycosaminoglycan matrix, for tissue repair are disclosed. Also disclosed are methods of using the described compositions for tissue repair.

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COMPOSITIONS AND METHODS FOR TISSUE REPAIR

BACKGROUND OF THE INVENTION

Mammals suffer tissue loss from a variety of mechanisms including trauma, tumor removal, vascular disease, genetic defects, cosmetic surgery and infections. Replacement of lost tissue or organs is often essential for either survival or function of the mammal.

A wide variety of implantable biomaterials has been used to repair tissue defects. Absorbable meshes made of polygalactin 910 (Vicryl™ (Ethicon Inc., Somerville, NJ)) and polyglycolic acid (Dexon™) can provide an intact structural repair, but lose tensile strength as they degrade (Yannas *et al., J. Biomed. Mater. Res. 14*:107-132 (1980); Kateusz *et al., PomeryW Medycynie 24*:3-39 (1994)). Once degraded, the fibrous tissue response that results does not have the strength to provide ongoing support of the repair and eventually breaks down. The incidence of recurrent herniation in the repair is nearly 100% (Green *et al., Surgery Gynecology & Obstetrics 176*:213-216 (1993)). For this reason, absorbable mesh cannot provide permanent reconstruction of load-bearing tissue.

Nonabsorbable structural meshes composed of polypropylene (PP) (e.g., Marlex™ (C.R. Bard Inc.), Prolene™ (Ethicon Inc., Somerville, NJ)), Dacron™ (e.g., Mersilene™ (Ethicon Inc., Somerville, NJ)) and expanded polytetrafluoroethylene (e.g., Gore-tex™ (W.L. Gore and Associates)) have generally been used for tissue repair. When applied in the form of a mesh, mechanical properties such as tensile strength, modulus of elasticity, and flexural rigidity can be controlled using a variety of polymers. PP mesh is the most commonly used prosthetic mesh for tissue defects, and it is ultimately the standard to which materials are compared. This macroporous mesh is inert, strong, and rapidly traversed by fibrous tissue. Scar tissue that forms around and through the mesh strengthens the repair zone. This tissue infiltration, however, is not well organized and the resulting scar tissue can contract and distort the mesh. Moreover, the outer ends of the mesh contain rigid monofilaments that are sharp and abrasive; these sharp edges have been reported to injure underlying viscera and erode through

overlying skin and soft tissue, leading to visceral perforation, fistulization, and infection. PP mesh also causes dense adhesions when it is placed adjacent to the abdominal viscera (Deguzman *et al., Endoscopy 27*:257-461 (1995)). Complications with the use of PP mesh include wound infection, scarring (Elliot *et al., Am. J. Surg. 137*:342-344 (1979)), seromas (Gilbert, *South Med. J. 80*:191-195 (1987)), sinus formation (Molloy *et al., Br. J. Surg. 78*:242-244 (1991); Boyd, *Surg. Gynecol. Obstet. 144*:251-252 (1977)), mesh extrusion (Voyles *et al., Ann. Surg. 194*:219-223 (1981); Lamb, *Surg. 93*:643-648 (1983)) and fistula formation (Talbert *et al., J. Pediatr. Surg. 12*:63-76 (1977); Deguzman *et al., Endoscopy 27*:257-461 (1995)).

Dacron™ mesh is more flexible than PP and rapidly conforms to anatomical defects. This mesh has not gained widespread use in the United States for several reasons. Dacron™ has been reported to elicit an inadequate fibrous response; several investigators have indicated that the fibrous tissue which grows into Dacron™ mesh becomes only loosely associated with the fibers of the mesh (Johnson-Nurse and Jenkins, *Biomaterials 10*(6):425-428 (1989)). Dacron™ also causes bowel adhesions and can cause visceral perforation and fistula formation. In addition, Dacron™ has a multifilament construction and has been associated with increased infection rates, as multifilament fibers provide an environment for bacteria to colonize which is relatively inaccessible to macrophages.

Expanded polytetrafluoroethylene is the least reactive of prosthetic materials and produces the least inflammatory response. The microporous structure is smooth, and, unlike PP and Dacron™, does not adhere greatly to abdominal viscera. However, this mesh does not become completely integrated into host tissue, and investigators have attributed a higher rate of recurrent hernias to this fact. The strength of repairs using expanded polytetrafluoroethylene are ultimately dependent on the strength of suture fixation between the edge of the tissue defect and the prosthetic component.

Many of the problems associated with permanent mesh for tissue reconstruction, such as lack of adequate fixation, adhesion, seroma/hematoma, fistula and scarring are related to the direct interaction of the mesh with the adjacent tissue. The presence of a foreign body in the would combined with the relatively

poorly vascularized scar tissue which surrounds the mesh also makes it susceptible to infection which can be difficult to eradicate without removal of the mesh. Thus, improved compositions for tissue repair are needed.

SUMMARY OF THE INVENTION

Structural biomaterials used to reconstruct abdominal wall defects restore abdominal wall integrity but may cause adhesions to the underlying abdominal viscera as well as additional problems. Work described herein demonstrates that integrating structural biomaterials with a biodegradable extracellular matrix analog reduced adhesions in an animal model. A composite mesh consisting of polypropylene (PP) integrated with collagen-glycosaminoglycan (CG), a biodegradable extracellular matrix analog, limits adhesion formation to viscera. The composite mesh (CG/PP) was implanted into surgically created ventral hernia defects (2 x 3 cm) in guinea pigs. Polypropylene mesh (PP) alone was used as a control. At 4 weeks, the abdominal wall was assessed for the degree of adhesions. Histologic examination of CG/PP mesh demonstrated the CG matrix to be partially to completely degraded, vascularized, and infiltrated with cells. The PP mesh, however, was surrounded by dense scar. The tissue layer that formed beneath the CG/PP mesh replaced the CG matrix and was thicker (0.34 ± 0.30 mm) than the scar layer of the PP repair (0.05 ± 0.02 mm). This vascularized tissue layer surrounded the polypropylene mesh and separated it from the underlying abdominal viscera, whereas PP mesh repairs allowed direct contact to the abdominal viscera. The percentage area of the repair covered with adhesions was significantly greater ($73 \pm 16\%$) in the PP mesh repair compared with that of CG/PP mesh ($20 \pm 15\%$) ($p = 8.2 \times 10^{-9}$). The adhesions which did occur with CG/PP mesh were almost exclusively to the omentum only whereas PP mesh formed adhesions to both omentum and small bowel in 100% of repairs. Adhesion strength was graded from 0 to 3 and was found to be greater in PP repairs (3 ± 0) than in those using CG/PP (1.7 ± 0.5) ($p = 1.6 \times 10^{-8}$).

Thus, integrating structural biomaterials with a biodegradable extracellular matrix analog reduced adhesions in this animal model. The CG/PP mesh allowed the formation of a vascularized tissue layer surrounding the polypropylene mesh,

protecting underlying abdominal organs. This technology is useful in reconstructive surgery by reducing adhesion formation while maintaining the strength of permanent structural biomaterials.

The invention pertains to compositions comprising at least one structural biomaterial integrated with at least one biodegradable matrix. Alternatively the composition can comprise two or more different biodegradable matrices. The structural biomaterial can be an absorbable or nonabsorbable material. For example, the structural biomaterial can be polypropylene mesh (e.g., Prolene™ (Ethicon Inc., Somerville, JN) and Marlex™ (C.R. Bard Inc.), Dacron™ (e.g., Mersilene™ (Ethicon Inc., Somerville, NJ)), silicone, polyethylene, polyamide, titanium, stainless steel, polymethylmethacrylate, silk, cotton, polygalactin, polyglycolic acid, Seprafilm™, poliglecaprone, collagen, gelatin, oxidized regenerated cellulose, polydioxone and expanded polytetrafluoroethylene (e.g., Gore-tex™ (W.L. Gore and Associates). The biodegradable matrix can be, for example, collagen glycosaminoglycan matrix (e.g., a crosslinked collagen glycosaminoglycan matrix), Gelfoam™ (Pharmacia and Upjohn, Inc., Kalamazoo, MI), Surgicel™ (Johnson & Johnson), Vicryl™ mesh (Ethicon Inc., Somerville, NJ) or Dexon™ mesh. The composition can also comprise a temporary optional moisture barrier to prevent evaporation and provide protection from the environment until sufficient epithelial coverage is obtained. The present invention also relates to synthetic tissue comprising a composition according to the invention.

In a particular embodiment, the structural biomaterial is disposed between a first and a second layer, each layer comprising at least one biodegradable matrix. In one embodiment, the biodegradable matrix comprising the first layer is different from the biodegradable matrix comprising the second layer. In a further embodiment of the invention, the first and second layer are larger than the structural biomaterial, i.e., the biodegradable matrix and not the structural biomaterial is in contact with the surrounding tissue.

The invention also pertains to compositions comprising at least one structural biomaterial integrated with at least one biodegradable matrix, wherein at least one biodegradable matrix has been seeded with cells. This technique can be used to produce a mature, differentiated, specific epithelium from any tissue from which

epithelial cells can be isolated and, optionally, cultured. For example, the cultured cells can be epithelial cells and mesenchymal cells (e.g., fibroblasts), as well as site-specific cells which have a specialized function, e.g., chondrocytes. In one embodiment, the cultured cells are grown to subconfluence in the culture medium.

When compositions according to the invention are used for tissue repair, adhesion to surrounding tissue is reduced as compared with a structural biomaterial alone. Furthermore, the use of compositions of the invention for tissue repair enhances formation of vascularized mesenchymal tissue as compared with a structural biomaterial alone. Thus, the invention also pertains to the use of compositions described herein for the repair of tissue in a mammal (e.g., a human). The tissue can be, for example, structural tissue, uroepithelium (e.g., bladder, urethra, ureter), gastrointestinal mucosa (e.g., oropharynx, esophagus, stomach, intestine), respiratory epithelium (e.g., trachea, bronchus) and vasculature (e.g., artery, vein, lymphatics). In a particular embodiment, the tissue repair is a hernia repair, such as an intestinal or abdominal hernia repair.

The invention also relates to a method for repairing tissue comprising the steps of applying a composition comprising at least one structural biomaterial integrated with at least one biodegradable matrix to an area to be repaired, such that the biodegradable matrix is interior to the defect; and securing the composition to the tissue surrounding the area to be prepared. In this method, adhesion of the composition to surrounding tissue is reduced as compared with repair using a structural biomaterial alone. Furthermore, formation of vascularized mesenchymal tissue is enhanced as compared with repair using a structural biomaterial alone. The repaired or regenerated tissue has a structure and function similar to that of the lost native tissue.

DETAILED DESCRIPTION OF THE INVENTION

Mammals suffer tissue loss from a variety of mechanisms including trauma, tumor removal, vascular disease, genetic defects, cosmetic surgery and infections. Replacement of lost tissue or organs is often essential for either survival or function of the mammal.

Many mammalian tissues can be thought of as bi-layer constructs. The surface layer contacts the environment or one or more body fluids, and the stromal layer provides mechanical support and a vascular supply to the surface layer(s). These bilayer tissue types include skin, trachea, bronchi, vermillion, oral lining, nasal lining, stomach, intestines, biliary ducts, ureters, bladder and blood vessels. When replacing any of these tissues or structures, it is essential that both the stromal and surface layers be reconstituted. In the examples below, abdominal hernia repair will be described in detail as an example of a tissue repair. However, this invention is not construed to be limited to abdominal or intestinal tissue as the appropriate tissue; the invention is intended to encompass any and all of the tissue constructions known in the art, particularly bi-layer tissues.

A mammal who has suffered extensive tissue loss or injury is immediately threatened by infection and by excessive loss of fluids. To meet both of these needs, a large wound must be closed promptly by some type of membrane. The most direct method of accomplishing this purpose is to remove the injured tissue and graft a composition to the wound, restoring the function of the injured tissue.

Polypropylene mesh remains the most widely used implant for structural tissue repair, such as hernia repair, due to its favorable mechanical properties and biocompatibility. Although placing polypropylene mesh directly in contact with abdominal viscera is usually avoided, this may not be possible in some reconstructions. Tissue reaction to mesh can cause excessive scarring and promote adhesion formation to abdominal viscera. These may lead to serious complications, including bowel obstruction, perforation, and fistula formation.

Work described herein has reaffirmed the high incidence of adhesion formation reported using polypropylene mesh directly exposed to peritoneal contents for ventral hernia repair (Alponat *et al., Am. Surg. 63*(9):818-819 (1997); Cristoforoni *et al., Am. Surg.62*(11):935-938 (1996)). PP graft repairs in this study formed dense adhesions to both the omentum and bowel involving 73% of the mesh surface involved. By integrating a biodegradable matrix, e.g., CG matrix, into a structural biomaterial, e.g., polypropylene mesh, adhesion strength, surface area, and degree of bowel involvement was significantly reduced.

The ability of a CG matrix to induce the formation of tissue has been well studied in the dermis and nerve (Yannas *et al., J. Biomed. Mater. Res. 14*:65-81 (1980); Yannas et al., *J. Biomed. Mater. Res.* 14:107-132 (1980); Dagalakis *et al., J. Biomed. Mater. Res. 14*:511-528 (1980); Murphy *et al., Lab. Invest. 63*:305 (1990)). The results of work described herein indicate that a biodegradable matrix (e.g., a CG matrix) becomes infiltrated with adjacent cells and revascularized during its degradation, similar to the response when the matrix is used as a dermal replacement. After 4 weeks, the mesenchymal tissue layer between the PP/CG implant and peritoneal cavity contained undegraded collagen fibers, infiltrated cells, and new blood vessels. This layer prevented adhesions to abdominal viscera compared to the PP mesh control grafts. In addition, it effectively provided a protective tissue barrier between mesh and the abdominal contents. The degradation of the CG matrix, including gluteraldehyde crosslinked CG/PP matrices, was less complete at 28 days than when this matrix was used as a dermal replacement in guinea pigs (Talbert *et al., J. Pediatr. Surg. 12*:63-76 (1977)). The less rapid degradation rate may be the result of intraperitoneal as opposed to dermal location (Murphy *et al., Lab. Invest. 63*:305 (1990); Orgill *et al., Wounds 8*(5):151 (1996)).

Increasing collagen crosslink density by gluteraldehyde treatment decreases *in vivo* degradation rate (Harriger *et al., J. Biomed. Mater. Res. 35*(2):137-145 (1997)). Gluteraldehyde treated CG matrix formed a thicker tissue layer under polypropylene mesh, perhaps due to less rapid degradation and, therefore, a greater degree of cellular infiltration.

Additional potential benefits of the CG/PP mesh include improving the contour of tissue between skin and mesh, provision of a vascularized tissue bed ventral to the polypropylene mesh in the event of deficient skin coverage for skin grafting, and reducing seroma and hematoma formation by facilitating tissue infiltration within the dead space. In addition, modifications to the CG/PP mesh can be made which provide functional characteristics such as incorporating antibiotics, anti-adhesive substances, growth factors, angiogenic or hemostatic agents. Alterations in the composition of the CG matrix may affect the rate of adhesion formation. Other glycosaminoglycans such as heparin (Fukasawa *et al., Int. J. Fertil. 36*(5):296-301 (1991); Turkcapar *et al., Int. Surg. 80*(1):92-94 (1995)) or

hyaluronic acid (Alponat *et al., Am. Surg. 63*(9):818-819 (1997); Becker *et al., J. Am. Coll. Surg. 183*(4):297-306 (1996)) could enhance anti-adhesive properties of the composition.

As described herein, compositions comprising at least on structural biomaterial integrated with at least one biodegradable matrix have been developed and tested in a guinea pig model. As also described herein, a method of repairing or regenerating tissue has been developed which optimizes functional repair, isolates or separates the structural biomaterial from the underlying tissue or organs to minimize adhesion to surrounding tissue, enhances formation of a pronounced fibrovascular infiltration into the composition, and provides a vascularized tissue bed which rapidly and completely surrounds the structural biomaterial and readily supports grafted tissue, such as split thickness skin grafts. As used herein, terms are intended to have their art-recognized meaning unless otherwise defined.

According to the present invention, a structural biomaterial is integrated with a biodegradable matrix to produce a composition for tissue repair. The structural biomaterial can be any material that has the following characteristics. The composition and structure of the material must be such that it does not provoke a substantial immune response from the mammal in whom it is implanted. The material should be permanent and non-biodegradable, particularly for load-bearing tissue, as such materials lose tensile strength as they degrade and the resultant fibrous tissue does not have the strength to provide ongoing support of the repair. For non-load-bearing tissue, the material can be biodegradable but should preferably persist for a period of time sufficient for the formation of a new mesenchymal tissue. Characteristics such as pore size, strength, water permeability and flexibility can be used to select an optimal material for specific tissue repair or reconstruction. Such optimization is routine and is dependent upon the desired properties of the material and the tissue to be repaired. Desirable characteristics can be determined, for example, with reference to Scales, *Proc. Roy. Soc. Med. 26*:647 (1953).

Suitable structural biomaterials for use in the invention include, but are not limited to, absorbable meshes made of polygalactin 910 (e.g., Vicryl™ (Ethicon Inc., Somerville, NJ)), polyglycolic acid (e.g., Dexon™), poliglecaprone (e.g., Monocryl™), Seprafilm™, collagen, gelatin, oxidized regenerated cellulose and

polydioxone (PDS™ (Ethicon Inc., Somerville, NJ)), and nonabsorbable materials such as polypropylene (e.g., Marlex™, Prolene™ (Ethicon Inc., Somerville, NJ)), Dacron™ (e.g., Mersilene™ (Ethicon Inc., Somerville, NJ)), silicone, polyethylene, polyamide, titanium, stainless steel, polymethylmethacrylate (PMMA), silk, cotton and expanded polytetrafluoroethylene (e.g., Gore-tex™ (W.L. Gore and Associates)). The compositions of the invention can comprises one or more (i.e., at least one) structural biomaterial.

The biodegradable matrix can be a highly porous, fibrous lattice. The lattice serves as a temporary tissue substitute and template for new tissue formation, and when combined with structural biomaterials it directs the formation of mesenchymal tissue adjacent to and incorporated in the biomaterial. The adjacent tissue, which is formed from cellular infiltration, neovascularization and/or collagen deposition, is incorporated into the structural biomaterial by surrounding individual fibers. This orderly, well vascularized tissue grows around and through the structural biomaterial, providing strength, vascularity and a barrier layer of tissue (unlike scar tissue) to separate the structural biomaterial from surrounding tissue and organs. The tissue formation also fixes the structural biomaterial securely to the surrounding tissue.

The biodegradable matrix can be any structure that has the following characteristics. The composition and structure of the lattice must be such that it does not provoke a substantial immune response from the graft recipient. The lattice must be sufficiently porous to permit blood vessels and mesenchymal cells from healthy tissue below the wound to migrate into the lattice. As discussed herein, this migration is referred to as "infiltration" and is responsible for the generation of the new dermis. Appropriate matrices can also be selected on the basis of properties such as degradation rate, hemostatic ability, degree of neovascularization, cellular infiltration and scar formation attributed to a particular matrix. This optimization is routine in the art.

To facilitate the formation of mesenchymal tissue, the matrix should be biodegradable. This biodegradation must not proceed so rapidly that the matrix disappears before sufficient healing occurs, i.e. before sufficient mesenchymal tissue forms. Matrices that degrade too slowly impede cell migration and cause the

formation of a fibrotic layer of cells surrounding the matrix. A matrix which biodegrades after about fourteen to thirty days is preferable.

The lattice can be either synthetic or biological in origin. For example, cadaver dermis can be used as a "natural" or biological biodegradable matrix of the present invention. Synthetic biodegradable matrices are preferred, however. Suitable biodegradable matrices are available commercially, or can be prepared by known methods, including, but not limited to, collagen glycosaminoglycan (CG) matrix, Gelfoam™, Surgicel™, Vicryl™ mesh, Dexon™ mesh, (see U.S. Patent Nos. 4,418,691 (Yannas *et al.*); 4,458,678 and 4,505,266 (Yannas and Burke); and 5,273,900 (Boyce *et al.*)). A "CG matrix" or "CG lattice" is a highly porous lattice made of collagen and glycosaminoglycan. The CG matrix serves as a supporting or scaffolding structure into which blood vessels and mesenchymal cells migrate from below the wound, a process referred to as "infiltration". Infiltration is responsible for creating new mesencymal tissue which replaces the CG matrix as it biodegrades. Cells from undamaged tissue surrounding the edges of the wound migrate into CG matrix to create a new, vascularized tissue bed.

Function of the biodegradable matrix such as a CG matrix is likely to be influenced by other physiochemical properties such as the type of glycosaminoglycan (GAG) used, the concentration of GAG, the pore structure, the collagen density, and the ability of collagen to activate platelets. These properties can be optimized using routine methods known to the skilled artisan. Various forms of GAG which may be suitable for use in this material include chondroitin 6-sulfate, chondroitin 4-sulfate, heparin, heparin sulfate, keratin sulfate, dermatan sulfate, chitin and chitosan.

It is possible to control several parameters of the CG matrix (primarily crosslinking density, porosity and GAG content) to control the rate of biodegradation of the lattice. Specific conditions for forming a CG matrix suitable for use in the present invention are given in the Exemplification. However, the skilled artisan will know of other conditions for forming CG matrices with variations of the above-mentioned parameters which are similarly suitable for use in the present invention. In addition, certain applications of tissue regeneration may require matrices which degrade more slowly or more quickly. The skilled artisan

will be able to recognize applications where it is desirable to vary the properties of the CG matrix, and will be able to vary the parameters accordingly; the present invention is intended to encompass such variations in the CG matrix..

Although the work described herein specifically exemplifies CG matrices, the tissue regeneration compositions and methods of this invention are not limited to CG matrices. Other suitable fibrous proteins, polymeric molecules, biological compositions or sintered ceramics having appropriate properties can be used in the present invention, and such lattices and materials are within the scope of this invention.

Seroma and hematoma formation is common in hernia repair due to large cavities created by raising soft tissue flaps, which after closure lie directly adjacent to the structural biomaterial closure. The inner surface of these wound cavities leak serous fluid and ooze blood which lead to seroma and hematoma formation. By combining the structural biomaterial and biodegradable matrix as described herein, a well vascularized mesenchymal tissue layer is rapidly formed which completely surrounds the prosthetic material. Seroma and hematoma formation may be reduced by the formation of a vascularized tissue layer between the structural biomaterial and the subcutaneous tissue. As newly formed tissue surrounds the structural biomaterial, it protects the adjacent tissue from perforations, erosion of the biomaterial through the skin and soft tissue, scar and adhesion formation, and trauma leading to bleeding or fluid accumulation. Soft tissue contour can also be improved by providing a biodegradable matrix layer between the biomaterial and the skin.

The compositions of the invention can comprise two or more layers in accordance with the teachings herein. For example, at a minimum the composition comprises one layer of biodegradable matrix and one layer of structural biomaterial. The composition can also comprise three layers, wherein the structural biomaterial is disposed between ("sandwiched between") two layers of biodegradable matrix. One benefit to the three-layer composition is that the first layer provides the ability to separate the structural biomaterial from surrounding tissue, and the second layer provides a vasularized bed which will support grafted tissue, help fill dead space or contour irregularities. Grafting of tissue can be performed using any of several

methods known in the art. Additional layers of biodegradable matrix and structural biomaterial can also be incorporated as desired to improve the properties of the compositions. The biodegradable matrix layers can be the same or different. In a preferred embodiment, one or both of the biodegradable matrix layers is larger than the structural biomaterial layer to allow the matrix to surround the biomaterial and prevent the biomaterial from contacting the surrounding tissue. Compositions comprising structural biomaterials and biodegradable matrices can be constructed by methods described herein or by other methods known in the art.

The compositions of the invention can also be seeded (e.g., in the biodegradable matrix layer) with cells prior to or after reconstruction. These cells will proliferate to form a functional epithelium simultaneously during the formation of the mesenchymal layer, obviating the need to graft epithelium in a separate procedure. Cells can be uncultured or cultured (e.g., to confluence or subconfluence). As used herein, "confluence" is intended to mean a merged or non-discrete cell layer. As used herein, "subconfluence" is intended to mean cells or a cell layer which has not grown to a point of confluence and contains separate and distinct cells or cell aggregates. These cells can be autologous, allogenic or xenogenic.

Specific tissue types can be regenerated by selecting the type of cells seeded into the matrix. Suitable cells include epithelial cells (e.g., keratinocytes), mesenchymal cells (e.g., fibroblasts) and site-specific cells. Also, as used herein, "site-specific" cells are intended to mean cells that produce a site-specific phenotype, e.g., cells that produce specialized epithelial structures such as lips, bowel mucosa or bladder tissue. In addition, other types of cells can be co-seeded into the matrix to allow the mesenchymal tissue layer to have specialized function or functions. For example, chondrocytes could be used for cartilage production or endocrine cells could be seeded to provide hormone production. Accordingly, the type of cell which is seeded onto the matrix will vary according to the tissue to be replaced and can be readily ascertained by the skilled artisan. Methods for seeding biodegradable matrices are known in the art and can be found, for example, in U.S. Patent Nos. 4,418,691 (Yannas *et al.*); U.S. Patent No. 4,060,081; 4,458,678 and

4,505,266 (Yannas and Burke); and 5,273,900 (Boyce *et al.*) and U.S. Patent Application No. 08/717,815 (Attorney Docket No. BWH96-01).

In addition, cells being used to seed a matrix may undergo genetic manipulation or modification in order to prevent rejection or to change the cell's phenotype in some beneficial manner. Genetic manipulation or modification includes, but is not limited to, introducing genetic matter into the cells so that the protein gene product or products are expressed in sufficient quantities to cause the desired change in phenotype, for example as described by Lyerly and DiMaio (*Arch. Surg. 128*:1197-1206 (1993)), the teachings of which are incorporated herein by reference. An example of suitable genetic matter includes the gene encoding for a growth factor along with the requisite control elements, as described in Morgan *et al.* (*Science 237*:1476 (1987)), the teachings of which are incorporated herein by reference. Other examples of suitable genetic material include, but are not limited to, the E1A oncogene (Barrandon *et al.*, *Proc. Natl. Acad. Sci. USA 85*:4102 (1989)) and the *neo* gene (Flowers *et al.*, *Proc. Natl. Acad. Sci. USA 87*:2349 (1990)), the β-galactosidase gene and the hGH gene (Vogt *et al.*, *Proc. Natl. Acad. Sci. USA 91*:9307-9311 (1994)).

The compositions of the invention can also comprise an optional moisture barrier, such as an impermeable silicone surface layer, which can provide a temporary border or cutaneous reconstruction to prevent evaporation and provide protection from the environment while the epithelial layer is forming and becoming confluent. The optional moisture barrier is any composition which can serve as an outer surface to the matrix or biomaterial and must be capable of being manually removed at will from the matrix or biomaterial. Compositions suitable for use as a moisture barrier must also have the property of being semipermeable to the passage through the wound of fluids from inside the body and impermeable to microorganisms such as bacteria and viruses from outside the body. The moisture barrier layer may not be necessary for internal uses or applications in which the tissue or organ is not exposed to air, and thus it is optional in such applications. Silicone elastomers are suitable for use in the moisture barrier of the present invention.

The term "mammal" as used herein includes, but is not limited to, primates (e.g., humans), cows, sheep, goats, horses, dogs, cats, rabbits, guinea pigs, rats, mice or other bovine, ovine, equine, canine, feline, rodent or murine species.

Once the structural biomaterial/biodegradable matrix composition has been prepared and, optionally, seeded, the wound is readied for application of the matrix. Areas of tissue that have been destroyed or damaged are surgically removed to prevent them from interfering with the healing process. The entire area of dead and damaged tissue is excised, so that intact epithelial cells are present at the perimeter of the wound. The composition, with the optional moisture barrier, if present, away from the wound, is draped across the wound to avoid the entrapment of air pockets between the wound and the composition. The matrix is sutured or stapled to the wound using conventional techniques and then covered with a bandage.

After application of the composition to the wound, blood vessels and mesenchymal cells from underlying healthy tissue begin, as described herein above, the process of infiltration of the grafted matrix. Once the neodermis and neoepidermis have progressed sufficiently to the point where the replacement tissue can function to protect the body against infection or infiltration from micro-organism and moderate fluid passage, the optional moisture barrier (if present) is manually removed from the matrix. The wound can then be covered with a dry sterile gauze which is changed periodically.

For example, abnormal tissue can be intentionally (e.g., surgically) removed from an individual and new tissue can be produced in its place using this method. Alternatively, the method of the present invention can be used to produce new tissue in place of tissue which has been lost due to accident or disease.

The present invention has application to massively burned patients as well as to patients undergoing reconstructive surgery, tissue trauma, surgical resection, infection, chronic skin diseases and chronic wounds. The present invention will also be useful in the replacement of other specialized epithelial tissues in a variety of organ systems, including, but not limited to, bone, cartilage, oral mucosa, uroepithelial, gastrointestinal, respiratory and vascular. Tissue loss from malignancy, congenital or acquired disease and surgical removal can be replaced with tissue composed of the same specialized native cells. Specialized epithelial

tissue such as bladder, ureter, oral mucosa, esophagus, trachea, blood vessel and intestine often requires replacement or reconstruction after surgical excision. Currently, this tissue can only be replaced with prosthetic material or a section of autologous tissue from another location with similar functional characteristics, often from a different organ system. The use of prosthetic material is limited by its non-viability, lack of specialized function, immunologic reaction or rejection and increased risk of infection. Autologous tissue from a separate location is often used to replace tissue defects. For example, intestine can be used for esophageal replacement and bladder reconstruction, and urinary conduit can be used for ureter loss or bile duct replacement. Also, donor veins are used to replace arteries. Using autologous tissue for replacement requires a surgical procedure and tissue loss from an uninjured organ. In addition, the donor tissue often does not have the identical structural or function characteristics of the native tissue and suffers from lack of specific anatomic and physiologic function.

Compositions described herein can be used by the oncologic, trauma or reconstructive surgeon to replace tissue defects with a tissue composed of organ-specific cells identical to the native tissue, without the need to violate uninjured organs for donor tissue. Such tissue can be replaced after surgical resection for malignancy, disease or trauma. This method allows for replacement of various commonly lost tissues such as oropharyngeal, nasal and bronchial mucosa, lip vermillion, blood vessels, trachea, esophagus, stomach, small and large bowel, biliary ducts, ureter, bladder, urethra, periosteum, synovium, areolar tissue, chest wall, abdominal wall and vaginal mucosa. Structural defects such as ventral, inguinal and diaphragmatic hernias, replacement or augmentation of tendons, ligaments and bone and abdominal and thoracic wall reconstruction can also be repaired as described herein. The lattice or matrix is flexible enough to be molded into the appropriate shape or form and then secured to adjacent or contiguous uninjured tissue while tissue regeneration progresses.

The following Examples are offered for the purpose of illustrating the present invention and are not to be construed to limit the scope of this invention.

The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated herein by reference.

EXAMPLES

Materials and Methods

Preparation of CG/PP Grafts

Bovine hide collagen (Sigma Chemical Co., St. Louis, MO) 0.5% by weight was dispersed in 0.05M acetic acid and co-precipitated with chondroitin-6-sulfate (Sigma Chemical Co., St. Louis, MO). The co-precipitate was concentrated by centrifugation and excess acetic acid was decanted. Concentrated co-precipitate, 3 ml, was poured into 3 x 5 cm wells on a flat stainless steel freezing pan placed on a cooled (-30° C) shelf of a freeze-drier. After the first freeze cycle, polypropylene (PP) mesh (™®, Ethicon Inc., Somerville, NJ) (2 x 4 cm) was placed over the CG mesh and 3 additional ml of the CG co-precipitate was poured over the mesh. After a second freeze cycle performed at -30° C, the frozen composite was then sublimated at 200 milliTor to produce a highly porous matrix completely surrounding the PP mesh. The collagen fibers of the matrix were cross-linked using a 24 hour dehydrothermal treatment at 105° degrees C and 30 milliTorr. Additional cross-linking was performed in selected PP/CG composites using a 24 hour treatment with a 0.25 % gluteraldehyde solution in 0.05 M acetic acid. The composite mesh was then exhaustively dialyzed in sterile, de-ionized water and stored in sterile 70% isopropanol until use. Alcohol was removed immediately prior to implantation by sequential washing with phosphate buffered saline. The hydrated CG/PP graft was approximately 3 mm in thickness.

Animal Model

Animals were housed in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care (ALAC) and cared for under an approved institutional protocol. Forty female Hartley guinea pigs, 500-525 g, were used in the study. The animals were anesthetized using Halothane (2.5 mg %), oxygen (4 L/min), and nitrous oxide (3 L/min). Electric shears were used to

remove the hair from the abdominal wall, which was then prepped with betadine solution and draped sterilely. A 3 cm vertical midline incision centered between the xyphoid and pubis was made through the linea alba and peritoneum to expose the peritoneal cavity. A PP or CG/PP mesh was placed within the peritoneal cavity dorsal to the abdominal wall and peritoneum. Twelve animals were used for PP mesh implantation. Additionally, 14 animals were implanted with CG/PP composites which had been gluteraldehyde cross-linked, and 14 animals were implanted with CG/PP composites without gluteraldehyde cross-linking.

The edge of the abdominal wall defect was sutured directly to the edges of the implants with a running 5/0 nylon suture. This repair resulted in an elliptical fascial defect (3 x 1 cm) bridged by the implant with a 0.5 cm overlap at all edges. A running 5/0 nylon suture was used to close the skin and the incision was dressed with petroleum impregnated gauze and an elastic bandage. Dressings were removed at 7 to 14 days and wounds left open to air.

At four weeks, the animals were sacrificed and the entire abdominal wall was circumferentially incised to the peritoneal cavity to widely expose the repair site. The exposure was performed gently to avoid disturbing any adhesions to viscera or omentum. Photographs were taken and observations were scored in a blinded fashion. The structures adherent to the mesh were recorded. These included omentum, small intestine, large intestine, stomach and liver. The percent surface area of mesh involving adhesions was estimated by visual inspection. Adhesion strength was scored qualitatively from 0 - 3, with 0 = no adhesions, 1 = adhesions easily freed with gentle tension, 2 = adhesions able to be freed with blunt dissection and 3 = adhesions requiring sharp dissection to separate from graft site. A transverse section of full-thickness abdominal wall including attached viscera was fixed in 10% formalin, embedded in paraffin and sectioned for staining with hematoxylin and eosin. Results were expressed as mean $\pm$ standard deviation. Comparisons were made using an unpaired Student's t-test.

Results

There were three anesthesia-related deaths (2 in the PP group and 1 in the CG/PP group) and ten animals (1 in the PP group and 9 in the CG/PP group) were

excluded after these animals damaged the dressings and skin sutures resulting in skin dehiscence.

In the remaining animals (n=27), adhesions to the biomaterials at 4 weeks were significantly more in the PP mesh (n=9) group than in the CG/PP (n=18) composite group. Some omentum was adhered to both CG/PP and PP implants. Small bowel, however, was adherent to only 3 of the 18 PP/CG repairs but to 8 of 9 PP repairs. The average adhesion score was less in the CG/PP (1.7 ± 0.5) than the PP (3.0 ± 0.0) ($p = 1.6 \times 10^{-8}$). The amount of the biomaterial surface area covered by adhesions was also less with the CG/PP composite ($20 \pm 15\%$) than with the PP ($73 \pm 16\%$) ($p = 8.2 \times 10^{-9}$) (Table 1).

Histological examination of the CG/PP mesh at 28 days showed the polypropylene mesh surrounded with a partially degraded CG matrix layer. The CG matrix was infiltrated with cells and was vascularized. This vascularized, mesenchymal tissue layer expanded through the interstices of the mesh fibers, encasing the polypropylene mesh with a continuous tissue layer which extended below the PP mesh an average of 0.34 ± 0.30 mm.

Using PP implants, the polypropylene was directly in contact with the abdominal viscera with dense adhesions. A discontinuous layer of scar tissue formed between the polypropylene mesh and the abdominal viscera which was only $0.05 \text{ mm} \pm 0.02$ mm in thickness. In multiple locations along the mesh, the fibers were directly adjacent and adhered to bowel.

Of the 18 animals in the CG/PP group, 9 animals received CG/PP mesh which underwent additional collagen crosslinking with gluteraldehyde, and 9 received CG/PP composites without gluteraldehyde cross-linking. Both groups, gluteraldehyde treated and untreated, formed significantly fewer, less dense adhesions and a thicker tissue layer in comparison to PP repairs (Table 2). In non-gluteraldehyde crosslinked materials there was only 18 % average surface area involvement, adhesion grade of 1.8, and the thickness of the tissue below the mesh was 0.13 mm (Table 3). Within the CG/PP group, comparisons were made between gluteraldehyde crosslinked and non-gluteraldehyde crosslinked mesh repairs (Table 4). No significant difference was observed in adhesion grade ($p= 0.54$) or percent

surface area (p= 0.35) when these subgroups were compared to each other. The tissue layer that formed under the polypropylene, however, was thicker in gluteraldehyde crosslinked CG/PP composite mesh repairs (0.68 ± 0.14) with than those without gluteraldehyde crosslinking (0.13 ± 0.04), p= 0.018. Qualitatively, there were more residual CG matrix fibers observed in gluteraldehyde treated CG/PP mesh repairs at day 28.

TABLE 1. Comparison of PP and CG/PP Mesh

	PP (n=7)	CG/PP (n=8)	p- value
Surface area involved (%)	73 ± 16	20 ± 15	8.2×10^{-9}
Adhesion grade	3.0 ± 0.0	1.7 ± 0.5	1.6×10^{-3}
Thickness of tissue below mesh (mm)	0.05 ± 0.02	0.34 ± 0.30	8.0×10^{-3}

TABLE 2. Comparison of Gluteraldehyde crosslinked CG/PP to PP mesh

	PP	CG/PP crosslinked	p- value
Surface area involved (%)	73 ± 16	22 ± 19	1.5×10^{-5}
Adhesion grade	3.0 ± 0.0	1.6 ± 0.5	3.9×10^{-7}
Thickness of tissue below mesh (mm)	0.005±0.02	0.68±0.14	8×10^{-3}

TABLE 3. Comparison of Non-gluteraldehyde treated CG/PP to PP mesh

	PP	Non- Gluteraldehyde Crosslinked	p- value
Surface area involved (%)	73 ± 16	18 ± 10	1.9×10^{-7}
Adhesion grade	3.0 ± 0.0	1.8 ± 0.0	3.3×10^{-7}
Thickness of tissue below mesh (mm)	0.05 ± 0.02	0.13 ± 0.04	8.0×10^{-3}

TABLE 4. Comparison within CG/PP mesh group (Gluteraldehyde treated vs. untreated)

	Gluteraldehyde Crosslinked	Non-Gluteraldehyde Crosslinked	p- value
Surface area involved (%)	22 ± 19	18 ± 10	0.54
Adhesion grade	1.6 ± 0.5	1.8 ± 0.0	0.35
Thickness of tissue below mesh (mm)	0.68 ± 0.14	0.13 ± 0.04	0.02

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. A composition comprising at least one structural biomaterial integrated with at least one biodegradable matrix.

2. A composition according to Claim 1, wherein the structural biomaterial is selected from the group consisting of nonabsorbable and absorbable materials.

3. A composition according to Claim 2, wherein the structural biomaterial is an absorbable material and is selected from the group consisting of polygalactin, polyglycolic acid, poliglecaprone, Seprafilm™, collagen, gelatin, oxidized regenerated cellulose and polydioxone.

4. A composition according to Claim 3, wherein the structural biomaterial is selected from the group consisting of Vicryl™ Monocryl™, PDS™ and Dexon™.

5. A composition according to Claim 1, wherein the structural biomaterial is a nonabsorbable material and is selected from the group consisting of polypropylene mesh, Dacron™, silicone, polyethylene, polyamide, titanium, stainless steel, polymethylmethacrylate, silk, cotton and expanded polytetrafluoroethylene.

6. A composition according to Claim 5, wherein the structural biomaterial is polypropylene mesh.

7. A composition according to Claim 6, wherein the polypropylene mesh is selected from the group consisting of Prolene™ and Marlex™.

8. A composition according to Claim 5, wherein the structural biomaterial is Dacron™.

9. A composition according to Claim 8, wherein the Dacron™ is Mersilene™.

10. A composition according to Claim 5, wherein the structural biomaterial is expanded polytetrafluoroethylene.

11. A composition according to Claim 10, wherein the expanded polytetrafluoroethylene is Gortex™.

12. A composition according to Claim 1, wherein the biodegradable matrix is selected from the group consisting of collagen glycosaminoglycan matrix, Gelfoam™, Surgicel™, Vicryl™ mesh, Dexon™ mesh, laminated matrix, polyglycolic acid, polygalactin mesh and collagen gel.

13. A composition according to Claim 12, wherein the biodegradable matrix is a collagen glycosaminoglycan matrix.

14. A composition according to Claim 13, wherein the collagen glycosaminoglycan matrix is crosslinked.

15. A composition according to Claim 1, wherein the structural biomaterial is disposed between a first and a second layer, each layer comprising at least one biodegradable matrix.

16. A composition according to Claim 15, wherein the biodegradable matrix comprising the first layer is different from the biodegradable matrix comprising the second layer.

17. A composition according to Claim 15, wherein the first and second layer are larger than the structural biomaterial.

18. A composition according to Claim 1, wherein at least one biodegradable matrix has been seeded with cells.

19. A composition according to Claim 18, wherein the cells are selected from the group consisting of epithelial cells and mesenchymal cells.

20. A composition according to Claim 19, wherein the cells are grown to subconfluence.

21. A composition according to Claim 1, further comprising an optional moisture barrier.

22. A composition according to Claim 1 which, when used for tissue repair, reduces adhesion to surrounding tissue when compared with a structural biomaterial alone.

23. A composition according to Claim 1 which, when used for tissue repair, enhances formation of vascularized mesenchymal tissue when compared with a structural biomaterial alone.

24. A composition comprising polypropylene mesh integrated with collagen glycosaminoglycan matrix.

25. Use of a composition comprising at least one structural biomaterial integrated with at least one biodegradable matrix for tissue repair.

26. A method according to Claim 25, wherein the tissue is selected from the group consisting of structural tissue, bone, cartilage, oral mucosa, uroepithelium, gastrointestinal mucosa, respiratory epithelium, chest wall, abdominal wall and vasculature.

27. A method according to Claim 25, wherein the tissue repair is a hernia repair.

28. A method according to Claim 27, wherein the hernia repair is an abdominal or inguinal hernia repair.

29. A method for repairing tissue comprising the steps of:

a) applying a composition comprising at least one structural biomaterial integrated with at least one biodegradable matrix to an area to be repaired, such that the biodegradable matrix is interior to the defect; and

b) securing the composition to the tissue surrounding the area to be prepared.

30. A method according to Claim 29, wherein the tissue is selected from the group consisting of structural tissue, bone, cartilage, oral mucosa, uroepithelium, gastrointestinal mucosa, respiratory epithelium, chest wall, abdominal wall and vasculature.

31. A method according to Claim 30, wherein the tissue repair is a hernia repair.

32. A method according to Claim 31, wherein the hernia repair is an abdominal or inguinal hernia repair.

33. A method according to Claim 29, wherein adhesion to surrounding tissue is reduced when compared with repair using a structural biomaterial alone.

34. A method according to Claim 29, wherein formation of vascularized mesenchymal tissue is enhanced when compared with repair using a structural biomaterial alone.

35. A synthetic tissue comprising a composition comprising at least one structural biomaterial integrated with at least one biodegradable matrix.

36. A method according to Claim 25 which is performed to repair a tissue defect resulting from trauma, surgical resection or infection.

37. A method according to Claim 29 which is performed to repair a tissue defect resulting from trauma, surgical resection or infection.

5

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 99/21515

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L31/00 A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61F C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 08277 A (COLETICA ;ORLY ISABELLE (FR)) 21 March 1996 (1996-03-21) page 3, line 3 - line 25 page 5, line 9 -page 6, line 5 page 7, line 5 - line 32 page 9, line 8 - line 17 ---	1-5, 12-15, 17,22, 23, 25-33, 35-37
X	WO 96 07355 A (FUSION MEDICAL TECHNOLOGIES IN) 14 March 1996 (1996-03-14) page 4, line 29 -page 5, line 13 claims 9,11-14 --- -/--	1-3,5-7, 15,22, 23, 25-33, 35-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 January 2000	24/01/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Menidjel, R

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/21515

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 060 081 A (YANNAS IOANNIS V ET AL) 29 November 1977 (1977-11-29) abstract column 3, line 38 -column 4, line 27 column 8, line 60 - line 68 examples 2-4	1,2,5, 12-14, 22,23, 25,26, 29,30, 33-37
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X	EP 0 372 969 A (JOHNSON & JOHNSON PATIENT CARE) 13 June 1990 (1990-06-13) abstract page 3, line 21 - line 52	1-3,22
P,X	EP 0 913 162 A (ETHICON ENDO SURGERY INC) 6 May 1999 (1999-05-06) abstract column 2, line 14 - line 57 column 5, line 14 - line 41 column 6, line 31 -column 7, line 15	1-3,5-7, 15,22, 23, 25-33, 35-37

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/21515

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 99 /21515

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 29-34, 37 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/US 99/21515

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